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In vivo effect of 1,25-dihydroxyvitamin D_3 on phagocyte function in hemodialysis patients

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In vivo effect of 1,25-dihydroxyvitamin D_3 on phagocyte function in hemodialysis patients. 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] has been shown to modulate the immune function of monocytes and macrophages. Patients with end-stage renal disease (ESRD) on chronic hemodialysis treatment usually present a deficiency of this active form of vitamin D_3. The aim of this study was to investigate the effect of 1,25$(OH)_2D_3$ replacement therapy on phagocytosis, bactericidal capacity, and oxidative metabolism of peripheral blood polymorphonuclear leukocytes (PMNL) and monocytes (MN) in chronic hemodialysis patients. Phagocyte function tests were performed before and after four weeks of an oral replacement therapy with 0.5 μg/day of 1,25$(OH)_2D_3$ (Rocaltrol®). The superoxide (O_2^-) generation of monocytes, measured by cytochrome c reduction and lucigenin-enhanced chemiluminescence (CL) from patients receiving hemodialysis treatment was significantly diminished compared to healthy controls. After the replacement therapy with 1,25$(OH)_2D_3$ the O_2^- production showed a significant improvement, resulting in an increased cytochrome c reduction and lucigenin-CL response. The bactericidal capacity of MN was also impaired and exhibited a significant enhancement of their killing activity after the administration of 1,25$(OH)_2D_3$. On the other hand, the luminol-enhanced CL, which reflects the myeloperoxidase-dependent oxidative metabolism, and the phagocytic ability of MN was not affected by the hormone. The function of polymorphonuclear leukocytes (PMNL) from hemodialysis patients showed no impairment in the state of 1,25$(OH)_2D_3$ deficiency and the replacement of the hormone did not enhance their function. These results suggest that the deficiency of 1,25$(OH)_2D_3$ in patients with ESRD on chronic hemodialysis treatment may be responsible for an impaired monocyte function, which could be improved by an in vivo replacement of the hormone.

Infection is a frequent complication and a major cause of morbidity and mortality in patients with end-stage renal disease (ESRD) who are on regular dialysis treatment [1, 2]. Abnormalities of the immune system could partly contribute to an increased susceptibility for bacterial infections. Several alterations of leukocyte function in patients with ESRD have been demonstrated, including both impaired neutrophil and monocyte function [3]. A decreased chemotaxis and reduced production of reactive oxygen metabolites during the "respiratory burst" of polymorphonuclear leukocytes and monocytes have been reported [4, 5]. These functions seem to decrease further with duration of dialysis treatment [6].

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The secosteroid hormone 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] is the active metabolite of vitamin D_3 and plays an important role in calcium hemostasis and skeletal metabolism. Recently it has been shown that 1,25$(OH)_2D_3$ exhibits immunodulatory activities, interacting with lymphocytes and mononuclear phagocytes [7]. Peripheral blood monocytes and tissue macrophages show an enhancement of the oxidative metabolism after in vitro incubation with 1,25$(OH)_2D_3$ [8–11]. In addition, 1,25$(OH)_2D_3$ is capable of inducing monocytic differentiation in various human [12] and murine [13] leukemic cell lineages. Abnormal macrophage function with a decreased chemotaxis and phagocytosis has been demonstrated in vitamin D-deficient mice and could be corrected after in vitro and in vivo replacement of the hormone [14]. Similar phagocyte alterations have been shown in children with rickets [15]. Furthermore, an improvement of a diminished leukocyte chemotaxis has been reported in patients with ESRD on hemodialysis treatment after in vitro and in vivo administration of 1,25$(OH)_2D_3$ [16].

Patients with ESRD usually present a deficiency of active 1,25$(OH)_2D_3$ due to the loss of renoparenchymal function. The present study was undertaken to determine the effect of 1,25$(OH)_2D_3$ on phagocytosis and oxidative metabolism of polymorphonuclear leukocytes (PMNL) and peripheral blood monocytes (MN) after in vivo replacement therapy with oral 1,25$(OH)_2D_3$ (Rocaltrol®) in patients with ESRD on regular hemodialysis treatment.

Methods

Patients

Twelve patients (7♂, 5♀; mean age 56.4 ± 13.1 years) with chronic renal failure on stable hemodialysis (3 times per week for 4 hours) who were not receiving any form of vitamin D therapy were investigated. The underlying kidney diseases were chronic glomerulonephritis (5), membranous nephropathy (1), tubular interstitial nephritis (2), polycystic kidney disease (2), reflux (1), and analgetic nephropathy (1).

Patients with diabetic nephropathy or with a medication of steroids, NSAID or immunosuppressive drugs were excluded from the study. Furthermore, patients with clinical evidence of infection and operations in the last four weeks prior to the entrance into the study were also excluded.

The patients received 0.5 μg 1,25-dihydroxyvitamin D_3 (Rocaltrol®) per os daily for four weeks. Calcium containing phosphate binders were replaced where necessary by aluminium hydroxyde during the study to avoid hypercalcemia. Phagocyte function tests were performed in vitro before and after the treatment. Serum levels of 1,25$(OH)_2D_3$ and intact parathyroid hormone (iPTH) were determined in the Bioscienta Laboratories (Ingelheim, Germany).

Controls

Twelve sex- and age-matched healthy adult volunteers with normal renal function and without signs of infection in the last four weeks served as controls.

Blood collection and preparation of leukocytes

Venous blood was collected into heparinized (10 U/ml) syringes containing 6% dextran in 0.9% saline (wt/vol). In patients on hemodialysis blood was obtained prior to dialysis. After sedimentation at room temperature for 45 minutes, the leukocyte-rich plasma was centrifuged at 350 g for 10 minutes (+4°C), and the pellet was resuspended in 15 ml Hanks balanced salt solution containing 0.1% gelatin (GHBSS). Polymorphonuclear leukocytes and mononuclear cells were separated by gradient density centrifugation at 350 g for 20 minutes at +4°C over Ficoll-Paque (Pharmacia, Uppsala, Sweden) by a method described elsewhere [17]. The PMNL fraction was rendered erythrocyte free by hypotonic lysis with 0.2% sodium chloride for one minute, and isotonicity was restored with an equal volume of 1.6% sodium chloride. After washing the polymorphonuclear and mononuclear cells twice with GHBSS cells were counted in a automatic cell counter (Sysmex, K-1000, Toa, Kobe, Japan). Subsequently, differential cell counts were performed using May-Grünwald-Giemsa staining and showed 92 ± 2% PMNL. The mononuclear cell fraction contained 35 ± 5% monocytes (MN), when stained for the presence of unspecific esterase (LeucoGnost® EST-Set, Merck, Darmstadt, Germany). Cell viability was evaluated by Trypan blue exclusion and always exceeded 95%. Finally, cells were resuspended in GHBSS at a concentration of 5×10^6 PMNL or MN/ml.

Superoxide anion measurement

The generation of superoxide anions by phagocytes was measured by assessment of superoxide-dismutase (SOD)-inhibitable cytochrome c reduction according to the method of Babior, Kipnes and Curnutte [18].

In brief, two pairs of polypropylene tubes containing either PMNL or MN (5×10^5/ml), ferricytochrome c (type VI, Sigma; 90 μM) and PMA (50 ng/ml) were placed in a shaking incubator for 10 minutes at 37°C in the presence and absence of SOD (100 μg/ml; Sigma) in a final volume of 1 ml. Prior to the addition of PMA, reaction mixtures were preincubated for five minutes at 37°C in a water bath and at the end of the incubation period superoxide production was terminated by placing the tubes on ice. Leukocytes were removed by centrifugation at 4°C (350 × g, 10 min). The cell-free supernatants were used for determination of cytochrome c reduction by measuring the absorbance at 550 nm on a spectrophotometer (DU-62, Beckman Instruments, Munich, Germany). The probes containing SOD were employed as reference. Values from duplicate samples without SOD were averaged and results were converted in nanomoles of cytochrome c reduced per 10^6 cells per minute by using extinction coefficient $E_{550\ nm} = 2.1 \times 10^4\ M^{-1}\ cm^{-1}$.

Chemiluminescence (CL) assay

To assess the respiratory burst activity of PMNL and MN two CL assay systems were used [19]. Lucigenin was used as a chemilumigenic probe to detect superoxide production, and luminol was used to detect oxygen metabolites arising from myeloperoxidase activity. CL mixtures in transparent plastic scintillation vials contained 500 μl of PMNL or MN (5×10^5/ml in GHBSS), 50 μl of either 2.5 μM luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione; Sigma) or 250 μM lucigenin (10,10-dimethyl-9,9′ biacridinium-dinitrate, Sigma) and 50 μl phorbol myristate acetate (PMA, 50 ng/ml; Sigma) as the CL stimulus. Prior to the addition of the stimulus (PMA) all samples were preincubated for six minutes in the measuring chamber at 37°C and background CL (0.2 to 1.6 × $10^3/10^4$ cells) was recorded. After the addition of PMA counts were obtained every two minutes for 30 minutes by using an automatic luminescence analyzer (LB 950, Berthold, Wildbad, Germany). CL results were expressed as the number of peak cpm per 10^4 cells.

Bacterial strain and culture conditions

Staphylococcus aureus Cowan I, a protein A-rich strain, was used in this study (provided by Prof. Verhoef and Dr. Verbrugh, University of Utrecht, Department of Microbiology, The Netherlands). For each experiment overnight cultures of bacteria were used, grown in 5 ml Müller-Hinton broth (Heipha, Heidelberg, Germany) at 37°C in a shaking incubator. After washing three times with phosphate-buffered saline (PBS, pH 7.4), bacteria were resuspended in GHBSS to a concentration of 5×10^8 colony-forming units (cfu/ml) using optical density measurement at 560 nm. The results were confirmed by plate colony counts. For the phagocytosis assay, the bacteria were radiolabeled by addition of 20 μCi [^{3}H] adenine into the overnight culture.

Opsonins and opsinisation procedure

Human pooled serum (HPS) was obtained from 10 healthy donors and aliquots were stored at −70°C until use. Just prior to the use, serum was diluted to 10% in GHBSS. Bacterial suspension (0.1 ml containing 5×10^8 cfu/ml) was mixed with 0.9 ml of the 10% HPS and incubated for 30 minutes at 37°C. The suspension was centrifuged and the bacterial pellet resuspended in 1 ml GHBSS and kept on ice until use (preopsonized bacteria).

Bacterial killing assay

The bactericidal capacity of PMNL and MN was determined in an assay modified from Quie et al [20]. Quadruplicate tubes with 100 μl preopsonized bacteria (5×10^7 cfu/ml) and 100 μl PMNL or MN (5×10^6/ml) were constituted. To one pair of samples 2.5 ml ice-cold sterile water was added immediately after the mixtures were composed (zero-time). The other pair was placed in a shaking incubator at 37°C and stopped after 60 minutes by adding ice-cold water as described above.

Subsequently, 200-fold dilutions in sterile Aqua dest. were made and duplicate 100 μl samples were striked out on agar

Table 1. Blood biochemistry before and after $1,25(OH)_2D_3$ replacement therapy from patients with chronic dialysis treatment

		Patients on chronic dialysis treatment	
(Normal values)	Controls	Before $1,25(OH)_2D_3$ therapy	After $1,25(OH)_2D_3$ therapy
$1,25(OH)_2D_3$ (19–67 pg/ml)	36.7 ± 18.5	8.5 ± 2.6[e]	24.3 ± 14.5[b]
Intact PTH (1.0–5.1 pmol/liter)	2.5 ± 1.2	23.8 ± 29.8[d]	11.3 ± 16.8[c]
Calcium (1.90–2.60 mmol/liter)	2.29 ± 0.08	2.34 ± 0.16	2.55 ± 0.22[c]
Phosphate (0.68–1.68 mmol/liter)	1.00 ± 0.16	1.66 ± 0.50[e]	1.89 ± 0.55[a]
Alkaline phosphatase (60–200 U/liter)	94.6 ± 17.4	104.5 ± 27.3	108.0 ± 33.6
Serum creatinine (−1.4 mg/dl)	0.85 ± 0.17	9.7 ± 2.5[e]	9.8 ± 2.7
Urea *mg/dl*	32.1 ± 8.2	148.3 ± 34.5[e]	154.7 ± 40.7
Hemoglobin *g/liter*	14.3 ± 1.2	9.5 ± 1.4[e]	9.3 ± 1.6
Total WBC count ($\times 10^3/\mu l$)	6.2 ± 1.7	6.6 ± 1.7	6.2 ± 1.2

Data are mean ± SD
[a] $P < 0.05$ vs. pretreatment with $1,25(OH)_2D_3$
[b] $P < 0.001$ vs. pretreatment with $1,25(OH)_2D_3$
[c] $P < 0.0005$ vs. pretreatment with $1,25(OH)_2D_3$;
[d] vs. controls
[e] $P < 0.0001$ vs. controls

plates (Colombia blood agar, Heipha, Heidelberg, Germany). After incubation for 18 hours at 37°C colonies were counted and results are expressed as the percentage of colonies killed after 60 minutes of incubation (percentage decrease in the number of cfu after the incubation period).

Phagocytosis assay

The uptake of preopsonized radiolabeled bacteria by phagocytic cells was determined in an assay described previously by Peterson et al [21]. In duplicate samples, 100 μl of preposonized bacteria (5×10^7 cfu/ml) were mixed with 100 μl of PMNL or MN (5×10^6/ml) in polypropylene vials (Wheaton omni-vials, Zinsser Analytic, Frankfurt, Germany) and incubated for 60 minutes at 37°C in a shaking incubator. The final bacteria-to-phagocyte ratio was 10:1. Phagocytosis was stopped by adding 3 ml of ice-cold GHBSS to the mixture. Subsequently the nonphagocyte-associated bacteria were removed by three times washing with GHBSS and differential centrifugation (160 × g, 4°C, 5 min) [22]. The final cell pellet was solved in 2.5 ml of scintillation liquid (Xylol containing Quickszint 212, Zinsser Analytic, Frankfurt, Germany) and phagocyte-associated radioactivity was determined in a liquid scintillation counter (Beckman Instruments). Phagocytosis was expressed as the percentage of uptake of total added radioactivity, based on the average of two separate vials containing radiolabeled bacteria alone.

Statistical evaluation

Evaluation of the results was done by using Student's *t*-test for paired and unpaired data, respectively. Levels of $P \leq 0.05$ were regarded as statistically significant. Data were presented as mean ± SD.

Table 2. Effect of $1,25(OH)_2D_3$ therapy on superoxide (O_2^-) production of polymorphonuclear leukocytes (PMNL) and monocytes (MN) from patients with chronic dialysis treatment

	nmol cytochrome c reduction/10^6 cells/min		
		Patients on chronic dialysis treatment	
Cells	Controls	Before $1,25(OH)_2D_3$ therapy	After $1,25(OH)_2D_3$ therapy
PMNL	15.49 ± 6.68	13.08 ± 2.56[a]	14.66 ± 4.26[c]
MN	10.10 ± 2.98	6.72 ± 2.72[b]	8.96 ± 2.42[d]

Data are mean ± SD.
[a] NS ($P > 0.05$) vs. controls
[b] $P = 0.012$ vs. controls
[c] NS ($P > 0.05$) vs. pretreatment with $1,25(OH)_2D_3$
[d] $P = 0.026$ vs. pretreatment with $1,25(OH)_2D_3$

Results

Twelve patients with end-stage renal disease (ESRD) who were on regular hemodialysis treatment, and not receiving any form of vitamin D supplementation were included in the study. Polymorphonuclear (PMNL) and monocyte (MN) function tests were performed before and after a four week course of treatment with 0.5 μg 1,25-dihydroxyvitamin D_3 (Rocaltrol®) daily per os. Laboratory values obtained before and after the treatment are summarized in Table 1. Blood levels of $1,25(OH)_2D_3$ increased significantly after the replacement therapy with Rocaltrol® and reached normal values after the administration, but were still lower than those of controls. The blood calcium and phosphate concentration showed a significant increase, whereas the intact parathormone (iPTH) significantly decreased. Other laboratory parameters remained unchanged during the treatment course.

Superoxide production

The results of the superoxide (O_2^-) production of PMNL and MN in response to stimulation with PMA, measured by cytochrome c reduction, are shown in Table 2. The generation of superoxide by monocytes from patients receiving dialysis was significantly diminished compared to healthy controls (6.72 ± 2.72 vs. 10.10 ± 2.98 nmol/10^6 cells/min; $P = 0.012$). After the $1,25(OH)_2D_3$ therapy the O_2^- production by MN showed a significant increase in the patients, nearly reaching the levels of controls (8.96 ± 2.42 nmol/10^6 cells/min; $P = 0.026$). No significant correlation was seen between increase of cytochrome c reduction and serum levels of PTH ($r = -0.542$, $P = 0.106$), calcium ($r = -0.279$, $P = 0.435$) and phosphate ($r = -0.093$, $P = 0.799$).

The generation of 0_2^- by PMNL did not exhibit a significant increase after treatment with $1,25(OH)_2D_3$ (14.66 ± 4.26 vs. 13.08 ± 2.56, $P = 0.236$).

Chemiluminescence

MN from patients on dialysis treatment exhibited a significantly reduced lucigenin-enhanced CL response compared to that of healthy controls ($146.3 \times 10^3 \pm 59.7 \times 10^3$ cpm/10^4 cells vs. $209.2 \times 10^3 \pm 72.0 \times 10^3$ cpm/10^4 cells, $P = 0.04$). Their CL response showed a significant improvement after the therapy with $1,25(OH)_2D_3$ ($182.2 \times 10^3 \pm 44.5 \times 10^3$ cpm/10^4 cells, $P =$

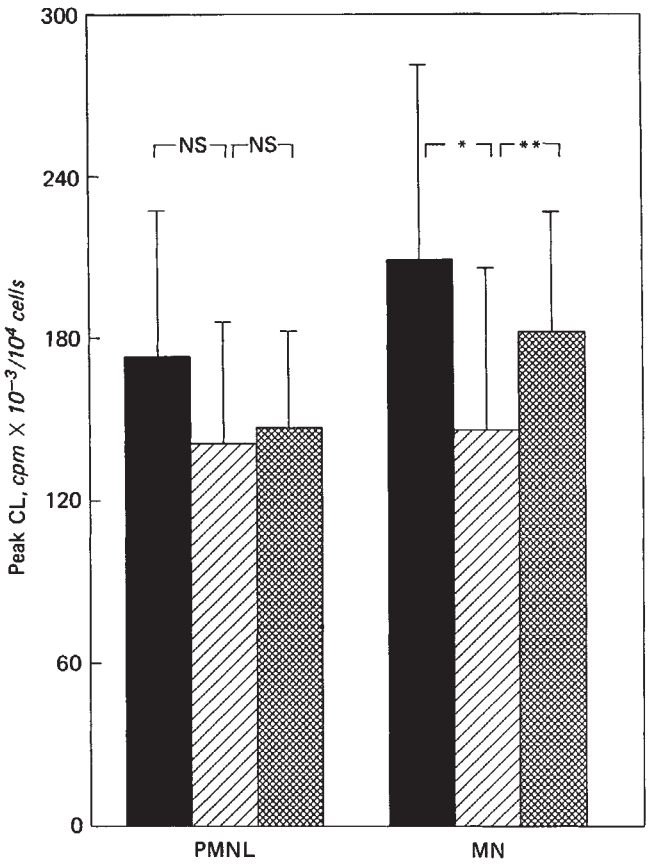

Fig. 1. *Lucigenin-enhanced chemiluminescence (CL) of PMA-stimulated polymorphonuclear leukocytes (PMNL) and monocytes (MN) from patients with chronic dialysis treatment before (▨) and after (▩) replacement therapy with $1,25(OH)_2D_3$ compared to healthy controls* (■). Data are mean ± SD; * $P = 0.04$, ** $P = 0.007$.

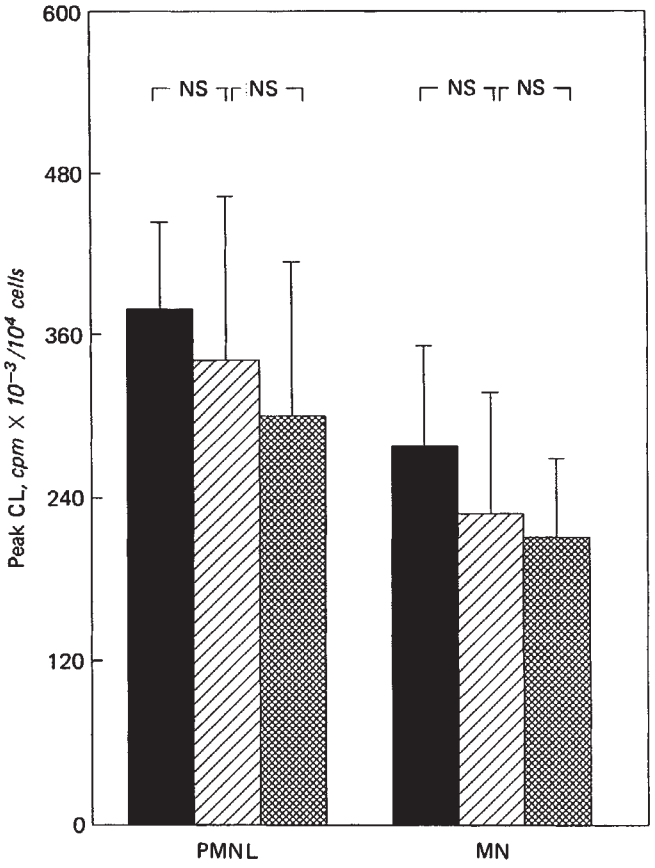

Fig. 2. *Luminol-enhanced chemiluminescence (CL) of PMA-stimulated polymorphonuclear leukocytes (PMNL) and monocytes (MN) from patients with chronic dialysis treatment before (▨) and after (▩) replacement therapy with $1,25(OH)_2D_3$ compared to healthy controls* (■). Data are mean ± SD.

0.007; Fig. 1). There was no significant correlation between the increased lucigenin-CL and serum levels of PTH ($r = -0.257$, $P = 0.474$), calcium ($r = -0.546$, $P = 0.103$) and phosphate ($r = -0.079$, $P = 0.828$).

The lucigenin-CL generation of PMNL was slightly, but not significantly reduced ($141.3 \times 10^3 \pm 44.6 \times 10^3$ cpm/10^4 cells vs. $173.4 \times 10^3 \pm 54.0 \times 10^3$ cpm/10^4 cells; $P = 0.127$) and remained unchanged after $1,25(OH)_2D_3$ supplementation ($147.3 \times 10^3 \pm 35.2 \times 10^3$ cpm/10^4 cells; $P = 0.524$).

The luminol-enhanced CL produced by PMNL and MN from patients after stimulation with PMA is shown in Figure 2. The peak CL response of PMNL and MN from patients receiving dialysis treatment did not differ significantly from those of controls (PMNL: $341.2 \times 10^3 \pm 121.2 \times 10^3$ cpm/10^4 cells vs. $378.6 \times 10^3 \pm 64.9 \times 10^3$ cpm/10^4 cells, $P = 0.356$; MN: $228.5 \pm 89.2 \times 10^3$ cells/10^4 cells vs. $278.4 \pm 73.0 \times 10^3$ cpm/10^4 cells, $P = 0.164$). Replacement therapy with $1,25(OH)_2D_3$ did not affect the luminol-enhanced CL from both PMNL and MN.

Bacterial killing

The killing capacity of *S. aureus* by MN from patients on dialysis was significantly reduced compared to that of healthy controls ($71.2 \pm 14.3\%$ vs. $84.3 \pm 6.7\%$, $P = 0.011$) and showed a significant improvement after $1,25(OH)_2D_3$ therapy ($71.2 \pm 14.3\%$ to $83.5 \pm 6.9\%$; $P = 0.019$). No significant correlation was seen between increase of killing capacity of MN and serum levels of PTH ($r = -0.144$, $P = 0.673$), calcium ($r = -0.158$, $P = 0.643$) and phosphate ($r = -0.418$, $P = 0.201$).

The ability of PMNL to kill *S. aureus* did not vary from that of controls and remained unchanged under treatment with $1,25(OH)_2D_3$ (Fig. 3).

Phagocytosis

The phagocytic ability (uptake of preopsonized *S. aureus*) of PMNL and MN from patients receiving chronic dialysis was similar to that of healthy controls and was not affected by $1,25(OH)_2D_3$ replacement therapy (Table 3).

Discussion

Recently, it has been demonstrated that the secosteroid hormone $1,25(OH)_2D_3$ is not only important for calcium hemostasis and bone metabolism, but also has immunodulatory properties [7]. It is well known that $1,25(OH)_2D_3$ induces the in vitro differentiation of leukemic and normal myeloid stem cells,

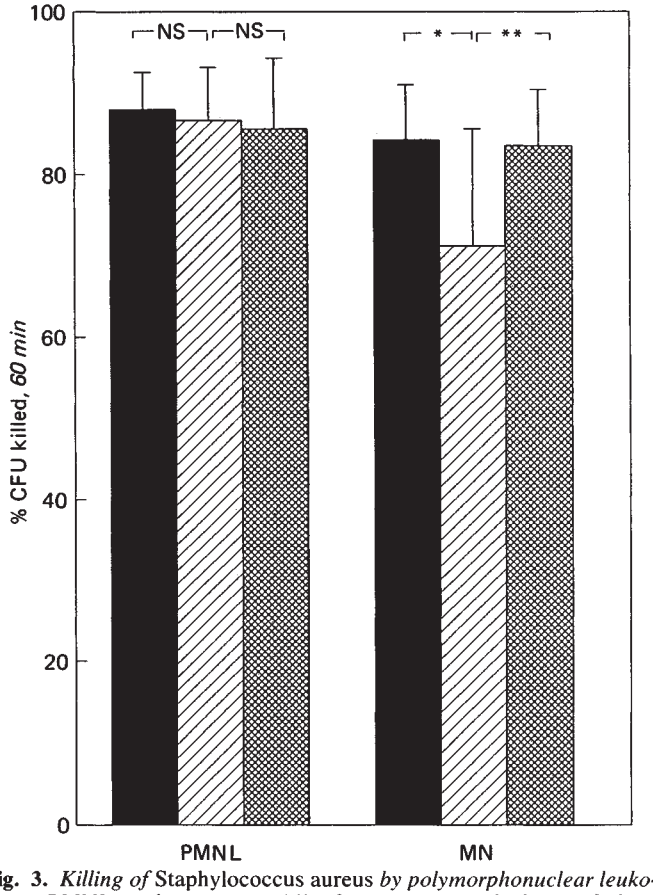

Fig. 3. *Killing of* Staphylococcus aureus *by polymorphonuclear leukocytes (PMNL) and monocytes (MN) from patients with chronic dialysis treatment before (▨) and after (▩) replacement therapy with $1,25(OH)_2D_3$ compared to healthy controls (■).* Data are mean ± SD; * $P = 0.011$; ** $P = 0.019$.

as well as that of peripheral blood monocytes toward macrophages [12, 23]. Furthermore, the hormone enhances the oxidative metabolism of peripheral blood monocytes (MN) and peritoneal macrophages (PMØ) in vitro, resulting in an increased production of hydrogen peroxide and superoxide [8–11] and an augmented killing of mycobacteria [24]. The action of $1,25(OH)_2D_3$ is mediated by a specific receptor on monocytes [25] and its expression increased after administration of $1,25(OH)_2D_3$ [26]. Consistent with these observations are the findings of an abnormal phagocyte function in vitamin D-deficient mice [14] and in humans with rickets [15].

Patients with end-stage renal disease (ESRD) on chronic dialysis treatment usually present a deficiency of $1,25(OH)_2D_3$, due to the loss of renoparenchymal function and inhibition of 1α-hydroxylase by hyperphosphatemia. Several alterations of leukocyte function have been demonstrated in these patients, including impaired phagocyte function [3]. Venezio et al [16] could demonstrate an impaired chemotaxis of PMNL and MN from patients on hemodialysis, which improved after therapy with $1,25(OH)_2D_3$.

The present study demonstrates that patients with ESRD on regular hemodialysis treatment showed markedly reduced serum levels of $1,25(OH)_2D_3$, and that their peripheral blood MN exhibited a diminished oxidative metabolism and killing activity. After the administration of Rocaltrol® normal serum values of $1,25(OH)_2D_3$ were obtained, which, however, were still lower than those of healthy controls. Furthermore, the MN from patients with ESRD on hemodialysis showed a significant improvement of their oxidative metabolism and bactericidal capacity after the in vivo replacement of $1,25(OH)_2D_3$.

Table 3. Phagocytosis of preopsonized *S. aureus* by polymorphonuclear leukocytes (PMNL) and monocytes (MN) from patients receiving chronic dialysis treatment under $1,25(OH)_2D_3$ therapy

	% Phagocytosis *60 min*		
		Patients on chronic dialysis treatment	
Cells	Controls	Before $1,25(OH)_2D_3$ therapy	After $1,25(OH)_2D_3$ therapy
PMNL	35.9 ± 5.5	36.9 ± 5.2[a]	36.6 ± 10.4[b]
MN	37.4 ± 6.4	38.9 ± 7.0[a]	40.1 ± 9.7[b]

Data are mean ± SD.
[a] NS ($P > 0.05$) vs. controls
[b] NS ($P > 0.05$) vs. pretreatment with $1,25(OH)_2D_3$

The PMA-stimulated superoxide (O_2^-) generation of MN, measured by cytochrome c reduction, was significantly lower in patients on dialysis than in healthy controls. After the replacement of $1,25(OH)_2D_3$ the production of O_2^- by MN reached values comparable with those observed in controls. In addition, the capacity of PMNL to generate O_2^- also showed a slight improvement, which, however, did not reach a significant level.

Previous studies have shown that peripheral blood MN as well as peritoneal macrophages (PMØ) from healthy donors exhibit an augmented PMA-induced H_2O_2 and O_2^- production after in vitro incubation with $1,25(OH)_2D_3$ [8–11]. Our results indicate that O_2^- generation by MN from patients on chronic hemodialysis treatment with $1,25(OH)_2D_3$-deficiency is diminished and can be improved after replacement of the active hormone. The mechanisms by which $1,25(OH)_2D_3$ enhances the cell's capacity for O_2^- generation is not well understood. Martell, Simpson and Taylor [27] showed that $1,25(OH)_2D_3$ stimulates the increase in protein kinase C (PKC) levels in leukemic promyelocytic (HL-60) cells. It is well known that PKC is activated by phorbol esters such as PMA and leads to cellular activation of phagocyte oxidative metabolism [28]. Furthermore, Wali et al [29] demonstrated recently that $1,25(OH)_2D_3$ stimulates membrane phosphoinositide turnover, activates PKC and increases cytosolic calcium in rat colonic epithelium. All these mechanisms are known to be involved in the activation of the leukocyte respiratory burst [30].

For further evaluation of the oxidative metabolism from phagocytes, we used two different CL assays. Lucigenin-enhanced CL was used because its light emission represents an additional, but more sensitive measure of the O_2^- production than the cytochrome c reduction assay [31, 32]. On the other hand, luminol-enhanced CL was used to detect oxygen metabolites arising from myeloperoxidase (MPO) activity. It had been shown that its light emission is dependent on the presence of MPO [33]. In accordance with the results obtained in the cytochrome c reduction assay, the lucigenin-enhanced CL response of PMA-stimulated MN from dialysis patients was

significantly lower than that of healthy controls. After $1,25(OH)_2D_3$ therapy the PMA-stimulated monocytes showed a significant rise in their lucigenin-amplified light emission. In contrast, the luminol-enhanced CL response of MN from patients on dialysis were slightly, but not significantly reduced compared to that of controls. After $1,25(OH)_2D_3$ therapy there was no significant change. This result suggests a normal MPO-dependent oxidative metabolism in these cells, which is not influenced by $1,25(OH)_2D_3$.

The PMNL response in the luminol-amplified assay from patients on dialysis treatment was also slightly but not significantly reduced. After the replacement of $1,25(OH)_2D_3$ no significant changes could be observed.

No significant changes were also observed by the determination of the phagocytic capability, measured by the uptake of preopsonized, radiolabeled *S. aureus*. Both PMNL and MN showed a normal activity compared to healthy controls, which was not altered by in vivo replacement of $1,25(OH)_2D_3$.

Furthermore, it could be demonstrated that the intracellular killing of *S. aureus* by MN from patients on hemodialysis is significantly diminished compared to healthy controls, and exhibits a significant improvement after in vivo replacement of $1,25(OH)_2D_3$. In contrast to our results, Venezio et al [16] described a normal killing capacity of MN both before and after $1,25(OH)_2D_3$ therapy. It is difficult to compare the results of these authors with ours, because they employed another assay system and determined the fungicidal capacity by using *C. albicans*. However, our findings are in accordance with several in vitro data describing an enhanced killing capacity of MN as well as PMØ after in vitro incubation with $1,25(OH)_2D_3$ [10, 11, 24]. The enhancement of MN's bactericidal capacity most likely reflects the stimulated oxidative metabolism with an increased generation of toxic metabolites, such as O_2^- which could be observed after $1,25(OH)_2D_3$ treatment. In addition, the bactericidal capacity of PMNL was found to be normal and did not change after the replacement of $1,25(OH)_2D_3$.

Another factor influencing the bacterial killing and superoxide production could be the increase of the serum calcium and phosphate concentration under treatment with $1,25(OH)_2D_3$. The data in the literature are conflicting with regard to the influence of calcium and phosphate metabolism on phagocyte functions. Previously, Carozzi et al [10] found a dose dependent increase of superoxide production and killing capacity after in vitro treatment of peritoneal macrophages (PMØ) with calcium concentrations between 0.5 and 3 mM in the medium, while calcium-free and 5 mM solutions suppressed these functions. On the other hand Khan et al [34] reported an inverse correlation between calcium levels, random migration and chemotaxis of PMNL from patients with primary hyperparathyreoidism. Doherty et al [35] found no influence of different calcium concentrations on the random migration of PMNL.

Hällgren et al [36] described an inverse relationship between serum phosphate levels and phagocytic ability of PMNL, whereas Cecchin et al [37] found no influence of anorganic phosphate on the phagocytic and killing activity of peritoneal PMNL. After $1,25(OH)_2D_3$ treatment we found a significant increase of serum calcium and phosphate levels, but when our data of superoxide production and killing capacity of MN were evaluated by regression analysis in relation to the increase of calcium and phosphate, no significant correlation was found. However, the increase of calcium concentration in our study was in a small range (mean 0.24 mM) in comparison to those concentrations used in the in vitro study of Carozzi et al [10]. This could be the reason that we did not find a correlation between the serum calcium levels and the increase of MN's killing capacity and superoxide production.

Previously Doherty et al [35] reported a significant inhibition of random migration of PMNL by prolonged exposure to PTH in patients with advanced renal failure. He showed a significant inverse relationship of random migration with serum level of PTH. However, we did not observe a significant change in the functional activity of PMNL, while the PTH level significantly decreased. Furthermore, we did not find a significant relationship between PTH level and the augmentation of oxidative metabolism and killing capacity of MN.

It is known that $1,25(OH)_2D_3$ also acts on activated T lymphocytes [25]. Thus, lymphocyte- and/or lymphokine-mediated effects may contribute to the observed changes in monocyte function. It has been previously described that $1,25(OH)_2D_3$ is a potent inhibitor of T-cell-proliferation as well as of interleukin-2 and interferon-γ production [38]. Both of these lymphokines are known to be potent stimulators of monocyte function. According to these findings one would expect a suppression rather than an stimulation of monocyte function under treatment with $1,25(OH)_2D_3$. Thus, it seems to be unlikely that lymphocyte-mediated effects are responsible for the observed enhancement of monocyte function.

In conclusion, superoxide generation and killing capacity of monocytes from patients with end-stage renal disease who are on hemodialysis treatment were diminished and showed a significant improvement after an in vivo replacement therapy of 1,25-dihydroxyvitamin D_3. Thus, the deficiency of 1,25-dihydroxyvitamin D_3 may be one of the factors contributing to the increased susceptibility for infections in patients with ESRD on dialysis treatment. The precise mechanisms modulating the enhancement of monocyte function by 1,25-dihydroxyvitamin D_3 are currently not yet understood and need further investigation.

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